

**AD-P008 753**



**ULTRASTRUCTURAL PATHOLOGY AND IMMUNOHISTOCHEMISTRY  
OF MUSTARD GAS LESION**

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**ABSTRACT**

The ultrastructural pathology of sulfur mustard gas (HD) skin toxicity has been characterized for several in vivo and in vitro model systems.<sup>1,2</sup> In animal models, the pathology involves the latent lethal targeting of skin basal cells, a disabling of hemidesmosomes and a progressive edema of the lamina lucida, all of which contribute to the formation of characteristic microblisters at the dermal-epidermal junction.<sup>1</sup> However, the effects of HD toxicity on structural proteins of extracellular domains of the dermal-epidermal junction have not been elucidated. We are beginning an immunohistochemical study of these domains in the hairless guinea pig and summarize here the time course effects of HD of three structural proteins: bullous pemphigoid antigen, laminin and Type IV collagen. The results of this combined ultrastructural and immunohistochemical study indicate that proteins of extracellular matrices of the basement membrane are antigenically altered during the development of HD-induced skin pathology and may contribute to the formation of microblisters.

**94-07915**



20030310248

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## INTRODUCTION

Immunohistochemical study of bullous diseases has been clinically useful for targeting skin structural proteins that are immunologically lost or altered to specific antisera. Proteins of the basement membrane zone now identified as affected in these diseases are bullous pemphigoid antigen, several hemidesmosomal proteins and specific proteins of the lamina lucida.<sup>3,4,5,6</sup> Two such lacydolytic bullous diseases which pathologically resemble sulfur mustard (HD)-induced skin lesion are bullous pemphigoid and junctional epidermolysis bullosa. Bullous pemphigoid is an acquired blistering disease primarily associated with the elderly.<sup>7</sup> The structural protein known to be immunologically altered in this disease is bullous pemphigoid antigen. Junctional epidermolysis bullosa is an inherited blistering disease which develops neonatally.<sup>8,9</sup> Glycoproteins of hemidesmosomes, anchoring filaments and of the lamina lucida are reported lost to specific immunoreaction in this bullous disease.

The ultrastructural pathology common to these bullous diseases and to sulfur mustard-induced skin lesion is the presence of characteristic microblisters or clefts which occur within the lamina lucida of the dermal-epidermal junction in the region of anchoring filaments. In the case of sulfur mustard, microblisters form as the result of the latent lethal targeting of basal cells, disabling of anchoring filaments of hemidesmosomes and a progressive inflammatory edema of the lamina lucida. However, the effects of HD toxicity on structural proteins of the microenvironment of the basement membrane zone and their contribution to the pathology have not been elucidated. We are beginning an immunohistochemical study of these domains in the hairless guinea pig, and present here the time course alteration of three structural proteins: bullous pemphigoid antigen, laminin and Type IV collagen.

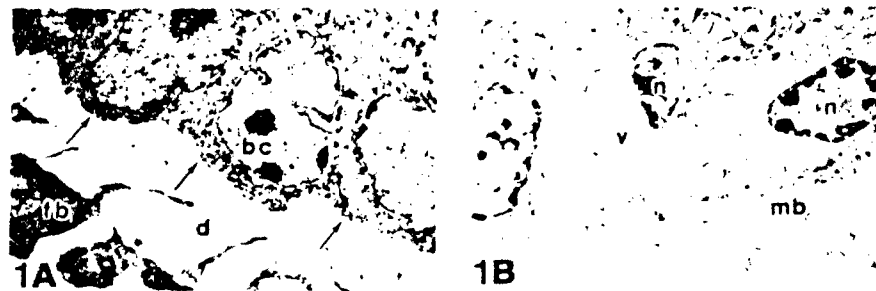
## METHODS

Skin sites of anesthetized hairless guinea pigs were exposed to the vapor of 10ul HD for 8 minutes and harvested at selected post exposure time periods of 6, 9, 12 and 24 hours.<sup>10</sup> Control sites were taken from non-exposed perilesional skin. Control and HD-exposed sites for light microscopic and ultrastructural study were immersion fixed for 24 hours in a cacodylate-buffered combined fixative of 1.6% formaldehyde and 2.5% glutaraldehyde. Following three washes in 0.1 M cacodylate buffer (pH 7.4, mOsm 190), light microscopy samples were processed for routine paraffin embedding while ultrastructural samples were postfixed in 1% osmium tetroxide for 1 hour, dehydrated in graded ethanol and embedded in epoxy resins. Semithin epoxy sections were differentiated with methylene blue, basic fuchsin and azure II for evaluation by light microscopy.<sup>11</sup> Ultrathin epoxy sections were nonspecifically counterstained with uranyl acetate and lead citrate for study by transmission electron microscopy. Fixed samples selected for scanning electron microscopy were critical-point dried and sputter coated with gold and palladium. Unfixed samples selected for immunohistochemistry were immediately plunge-frozen in liquid freon.

Cryosections, 12µm thick, were collected onto gelatin coated glass slides and air dried. Following a 5-minute wash in PBS (pH 7.6, mOsm 300), sections were then immunohistochemically incubated with working dilutions of specific antibody to bullous pemphigoid antigen (BPA), laminin and Type IV collagen according to the following antibody sequence: 3% normal goat serum (20 minutes), specific primary antisera (1 hour), peroxidase conjugated goat bridging antibody (30 min). Peroxidase conjugated antibody was cytochemically developed with diaminobenzidine. Myeloperoxidase of leukocytic infiltrates was also detected with diaminobenzidine. Cryosections not treated with specific primary antibody were used as method controls.

## RESULTS

Light microscopic and ultrastructural analysis revealed the typical progression of HD-induced basal cell pathology and the formation of characteristic microblisters at the basement membrane zone (Fig. 1).



**Figure 1.** Electron micrographs of basement membrane zone of hairless guinea pig skin. A. Non-exposed control skin with epidermal basal cell (bc), basement membrane (arrows) composed of the lamina lucida and basal lamina, and dermis (d) with fibroblasts (fb). B. HD-exposed skin with pyknotic nuclei (n) of basal cell, vacuolated cytoplasm (v) and microblister formation (mb) at the dermal-epidermal junction. Magnifications 4800X.

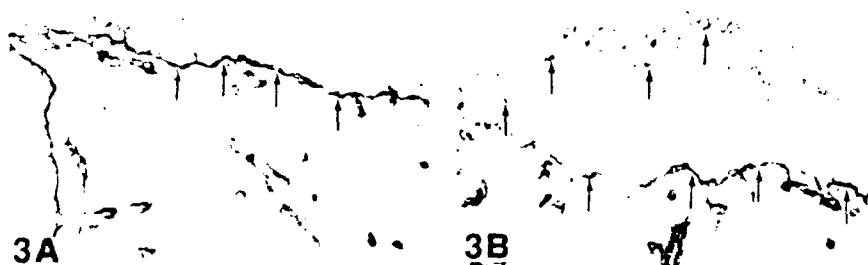
Basal cell pathology, beginning after a prevesication latency period of 6 hours, included progressive nuclear and cytoplasmic changes leading to cell swelling, degeneration, fragmentation and death. The targeting of basal cells was to the exclusion of other epidermal cells of all strata. Microblisters, first appearing at 12 hours post exposure, formed within the lamina lucida as the result of the lethal effects on the basal cell, the disabling of hemidesmosomes, a progressive edema at the lamina lucida and infiltration of leukocytes.

Companion immunohistochemical studies demonstrated that control sections were consistently immunoreactive for BPA at the lamina lucida most proximal to basal cell plasma membranes. However, following exposure to HD, BPA immunoreactivity was weak at early post exposure times and subsequently lost to specific antisera at later time periods (*Fig. 2*).



*Figure 2.* Light micrographs of immunohistochemically localized BPA in hairless guinea pig skin. A. Non-exposed control skin with BPA (arrows) localized to the lamina lucida. Magnification 330X. B. HD-exposed skin with microblister formation (mb) and the complete absence of immunoreactivity for BPA. Magnification 200X.

Laminin, while localized throughout the entire lamina lucida in control sections, was unaltered during the prevesication time periods post exposure. At later time periods, laminin reactivity was spotty and conformed to the now structurally altered lamina lucida at microblister lesion sites (*Fig. 3*).



*Figure 3.* Light micrographs of immunohistochemically localized laminin. A. Non-exposed control skin with laminin (arrows) localized throughout the lamina lucida. B. At a microblister of HD-exposed skin showing scanty localization of laminin (arrows). Magnification: 165X.

The reactivity of Type IV collagen was unaltered to specific antisera throughout prevesication and vesication time periods of these experiments.

## DISCUSSION

The proteins addressed in this study are those identified as adherent structural macromolecules of extracellular domains of the basement membrane zone.<sup>12</sup> BPA is a noncollagenous protein shared between basal cell hemidesmosomes and the lamina lucida. It has been used clinically for rapid immunofluorescent evaluation of bullous lesion boundaries and diagnostically may be absent or faintly localized in bullous pemphigoid and junctional epidermolysis bullosa patients.<sup>4,5</sup> Laminin, a noncollagenous glycoprotein of the lamina lucida has also been used to define boundaries of bullous lesions.<sup>4</sup> Type IV collagen is a ubiquitous protein assigned to the lamina densa of basement membranes. Its localization is useful in demarcating basement membranes.

In the present study, the loss of BPA antigenicity to specific antisera early during the prevesication period of HD toxicity suggests that this protein may be conformationally changed directly by the alkylating effects of HD. Since the association between altered BPA and bullous pemphigoid lesion is known, it may be predicted that an induced change of BPA antigenicity may subsequently affect its adherent properties as well as promote its candidacy as an autoimmune antigen. Laminin, altered to recognition only during the vesication period, may be responding to released proteases from the toxic basal cell, to other cellular chemical mediators of the inflammatory response or both. Type IV collagen, remaining immunospecifically intact throughout the time course of the toxicity, correlates with the apparently maintained structural integrity of the lamina densa during the development of the vesication as demonstrated by histopathological and ultrastructural study.<sup>1,13</sup>

## CONCLUSION

The results of this combined ultrastructural and immunohistochemical study suggest that proteins of the extracellular matrices of the basement membrane zone microenvironment are affected during the development of HD-induced skin pathology. Still to be investigated is the role of this alteration in the pathogenesis of characteristic microblisters, its influence on repair mechanisms following HD toxicity and its possible use as a diagnostic strategy in predicting HD vesicating lesions.

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